# PATENT COOPERATION TREATY

# PCT



## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br>PU0404-PCT | **FOR FURTHER ACTION** See Form PCT/IPEA/416 | |
|---|---|---|
| International application No.<br>PCT/SE2005/000085 | International filing date *(day/month/year)*<br>26-01-2005 | Priority date *(day/month/year)*<br>29-01-2004 |
| International Patent Classification (IPC) or national classification and IPC<br>See Supplemental Box | | |
| Applicant<br>Amersham Biosciences AB et al | | |

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 24-08-2005 | 24-03-2006 |
| Name and mailing address of the IPEA/SE<br>Patent- och registreringsverket<br>Box 5055<br>S-102 42 STOCKHOLM<br>Facsimile No. +46 8 667 72 88 | Authorized officer<br>Ida Christensen/Els<br>Telephone No. +46 8 782 25 00 |

**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Cover sheet

**International patent classification (IPC)**
***G01N 30/72*** (2006.01)
***B01D 15/08*** (2006.01)

**INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY**

International application No.
PCT/SE2005/000085

**Box No. I Basis of the report**

1. With regard to the **language**, this report is based on:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____ ,
which is the language of a translation furnished for the purposes of:

- ☐ international search (Rules 12.3(a) and 23.1(b))
- ☐ publication of the international application (Rule 12.4(a))
- ☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-8 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* 1-3 received by this Authority on 15-02-2006
pages* _____ received by this Authority on _____

☒ the drawings:

pages 1-5 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

**INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY**

International application No.
PCT/SE2005/000085

**Box No. V** **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-17, 20-23 | YES |
| | Claims | 18, 19 | NO |
| Inventive step (IS) | Claims | 1-17 | YES |
| | Claims | 18-23 | NO |
| Industrial applicability (IA) | Claims | 1-23 | YES |
| | Claims | | NO |

2. Citations and explanations (Rule 70.7)

The present application relates to a method for reducing the complexity of a biological sample and a system for performing said method. The complexity is reduced by selecting a fraction from the entire native or digested biological sample after a first separation (e.g. by anion exchange chromatography (AEC), isoelectric focussing or chromatofocussing), said fraction containing peptides which have a pI-value within a limited range and which fraction represents a subset of or the entire substance population in the sample. Said fraction is separated further by a second separation (e.g. cation exchange chromatography (CEC)). Thereafter, the separated components are analysed by mass spectrometry (MS).

Reference will be made to the following documents cited in the International Search Report:

D1) US 5416023
D2) Nature Biotechnology, 19:242-247 (2001), Washburn et al.
D3) Electrophoresis, 23:3143-3148 (2002), Chen et al.
D4) J Chromatog B, 787:11-18 (2003), Wang & Hanash

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**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

D1 discloses a system comprising a column combination comprising an anion exchange medium, a cation exchange medium and a reverse-phase medium (see claim 1).

The system according to claims 18-19 lacks novelty. It is defined by its components and does not obtain novelty merely due to its field of application.

The system according to claims 20-23 is novel.

D1 is considered to represent the closest prior art.

The system of claim 21 differs from what is disclosed in D1 in that the charge-selective column is a chromatofocussing column instead of an anion exchange column.

The system of claim 22 differs from what is disclosed in D1 in that the charge-selective column is an isoelectric focussing column instead of an anion exchange column.

However, said differences are not considered to represent solutions which involve an inventive step. It is obvious for the person skilled in the art to construct a system comprising a chromatofocussing column or an isoelectric focussing column instead of an anion exchange column. All components of the system are previously known in the art.

Consequently, the system according to claims 21-22 is considered to lack inventive step.

The system according to claims 20 and 23 differs from what is known from D1 in that the pH-values of the buffers used for the charge-selective column and the cation exchange column, respectively, are described.

However, said differences are not considered to represent solutions which involve an inventive step. The system according to claims 20 and 23 is not considered to be sufficiently adapted to the method of claims 1-17. It is doubted that the system contains all technical features which are needed in order to perform the method successfully. Therefore, the system of claims 20 and 23 is considered to lack inventive step.

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**Supplemental Box**

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

D2 describes a large-scale analysis of the yeast proteome by multidimensional protein identification technology (see page 246, column 2, paragraphs 3-5).

D3 relates to the use of capillary isoelectric focusing and capillary reversed-phase liquid chromatography for two-dimensional proteomics separation (page 3144, column 2, paragraph 2; page 3145, column 1, paragraph 1; page 3147, column 1, paragraph 1).

D4 describes multi-dimensional liquid phase based separations in proteomics (see the entire document).

Documents D2, D3 and D4 represent prior art and are not considered to be relevant for the assessment of novelty and inventive step of the method according to claims 1-17 or the system according to claims 18-23.

The method of claims 1-17 is novel and involves an inventive step.

The subject-matter of claims 1-23 is industrially applicable.

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## AMENDED CLAIMS

1. A method for reducing total sample complexity in native or digested biological sample(s), before analysis thereof by mass spectrometry, comprising the following steps:

a) selecting a fraction from the entire native or digested biological sample(s) on the basis of pI-value, said fraction comprising native or digested sample representing the entire substance population in the sample;

b) separating native or digested sample substances from each other; and

c) analysing said substances by mass spectrometry.

2. A method according to claim 1, wherein said substances are peptides obtained from proteins in the sample(s).

3. A method according to claim 1 or 2, wherein the pI-value is 3.5 - 4.5 or a sub range thereof.

4. A method according to claim 1 or 2, wherein the pI-value is selected to target one or more specific peptides.

5. A method according to one or more of the above claims, wherein said fraction in step a) is obtained by anion exchange chromatography.

6. A method according to claim 5, wherein the separation in step b) is by cation exchange chromatography.

7. A method according to one or more of the above claims, wherein, in step a), the sample is dissolved in a buffer with pH 4.5, the sample is loaded onto an anion exchange column, and the desired peptides are eluted in a buffer with pH 3.5.

8. A method according to one or more of the above claims, wherein the separation in step b) is by multidimensional chromatography, MDLC, comprising cation exchange chromatography, RPC (reverse phase chromatography) and MS/MS.

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9. A method according to one or more of the above claims, wherein the anion exchange column is coupled to the cation exchange column.

10. A method according to claims 8 or 9, wherein the pH in step a) is higher than in step b).

11. A method according to any of the claims 1-4, wherein the fraction in step a) is obtained by isoelectric focussing.

12. A method according to any of the claims 1-4, wherein the fraction in step a) is obtained by chromatofocussing.

13. A method according to claim 11 or 12, which is integrated to a conventional MDLC (multidimensional liquid chromatography) flow path.

14. A method according to one or more of the above claims, wherein the mass spectrometric analysis is tandem MS.

15. A method according to one or more of the above claims, wherein the MS is ESI (electrospray ionisation)-MS.

16. A method according to one or more of the claims 1-14, wherein the MS is MALDI (matrix assisted laser desorption ionisation)-MS.

17. A method according to one or more of the above claims, wherein the biological sample(s) comprises at least two samples which are differentially labelled.

18. A system for reducing total sample complexity in a method according to one or more of the claims 1-17, comprising a charge-selective column coupled to a MDLC work flow path comprising a cation exchange column and a RPC column.

19. A system according to claim 18, wherein the charge-selective column is an anion exchange column.

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20. A system according to claim 18 or 19, wherein the charge-selective column is run with a first buffer having pH 4.5-4.0 and a second buffer having pH 3.5-4.0, wherein the second buffer has lower pH than the first buffer and is used for elution.

21. A system according to claim 18, wherein the charge-selective column is a chromatofocussing column.

22. A system according to claim 18, wherein the charge-selective column in an isoelectric focussing column.

23. A system according to one or more of the claims 20-22, wherein the cation exchange column is run with a third buffer with pH lower than the buffer used for elution from the charge-selective column.